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RESEARCH ARTICLE

Survival of the copepod *Mesocyclops longisetus* during simulations of transport from hatchery to target areas for biological control of mosquito larvae

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ABSTRACT. Copepods have been successfully used in many countries for the biological control of larvae of mosquitoes that vector diseases. In Brazil, this line of research has been focused on the use of the copepod *Mesocyclops longisetus* (Thiébaud, 1914) for the biological control of the mosquito *Aedes aegypti* (Linnaeus, 1792). The transportation of the copepods from the place where they are reared to where they will be used often involves long distances for extended periods of time. This study assesses the survivorship of *M. longisetus* during simulation of transport under different conditions. Different loading densities (20, 30, 40, 80, and 120 ind.L) and stirring times (30 minutes, one hour, two hours, and four hours) were tested. Survivorship was high, with 75% of the results equal or higher than 90% survival. Reduced mortality was observed when transportation time was up to 120 minutes and densities were up to 40 ind.L. In higher densities or longer transportation times, the mortality rate was significantly affected.

KEY WORDS. *Aedes aegypti*, Copepoda, Culicidae, density.

INTRODUCTION

The mosquito *Aedes aegypti* (Linnaeus, 1792) is a vector of viruses that cause several globally important diseases, such as dengue fever, yellow fever (Braga and Valle 2007), and Chikungunya (Vega-Rúa et al. 2014). These diseases can be controlled by reducing mosquito populations. Different approaches are adopted worldwide: mechanical, chemical, and biological control, in addition to educational activities (Wermelinger and Ferreira 2013). In Brazil, dengue affects hundreds of people annually, resulting in many fatalities. The control of *A. aegypti* populations involves the elimination of standing water through the destruction of potential sites of mosquito proliferation, and the use of insecticides and larvicides (Brasil 2001).

The chemical control of mosquitoes using insecticides and larvicides raises concerns for the health of the people who have to apply the products (Lima et al. 2009), damages to the environment (Fournet et al. 1993), and selects for resistant mosquito strains (Andrade and Santos 2004). An alternative currently adopted in many parts of the globe is the biological control of mosquito larvae using copepods (French Polynesian islands: Rivière et al. 1987, Lardeux et al. 1992; in New Orleans, US: Marten 1990; Honduras: Marten et al. 1994). In Brazil, these biological control efforts are still experimental and are focused on the copepod species *Mesocyclops longisetus* (Thiébaud, 1914). This species has been recognized as an effective predator of *A. aegypti* larvae (Nam et al. 1998, 2012, Panogadia-Reyes et al. 2004, Baldacchino et al. 2015) and has the advantage that it is native to Brazil.

The technology for the large-scale cultivation of *M. longisetus* is already available (Marten et al. 1997, Andrade and Santos 2004). However, the implementation of biological control in Brazil still needs further research. For instance, when copepods are produced in places that are far from the control target areas of *A. aegypti*, they need to be transported. The results of this study may help in the control of mosquito larvae, especially in the most affected areas.

MATERIAL AND METHODS

Copepods were reared in one-liter plastic containers. The containers were filled with mineral water fertilized with 0.02 grams per liter of NPK (10:10:10). The goal of the fertilization was the creation of a phytoplankton biomass to feed the copepods. The transport was simulated by fulfilling 1.5- and two-liter plastic bottles with a mixture of culture medium containing copepods and mineral water (same proportion 1:1) and stirring the bottles using an orbital shaker model SL-180/A (2.5 shakes per second). Different copepod densities were tested: 20, 30, 40, 80, and 120 ind.L. The transport times varied from 30 minutes to one hour, two hours, and four hours. All treatments were tested in triplicates. Copepod survivorship was estimated after a resting period of 12 hours. Survivorship of the control treatment, consisting of three bottles that were not stirred, was also estimated for each of the tested densities.

The effect of copepod density and transport simulation time (explanatory variables) versus copepod survival (response variable) were evaluated separately. First, we evaluated the effect of transportation duration versus copepod survivorship separately for each copepod density, and then the effect of copepod density versus copepod survivorship separately for each transport duration. After that, the combined effect of the explanatory variables was evaluated through multiple linear regression analysis. The analyses were performed using R Cran Project 3.3.0 software (2016).

RESULTS

Mean copepod survivorship after transport simulations was 95%, ranging from 70 to 100%. Additionally, 75% of the results were equal to or higher than 90% survival. The mortality of copepods that were not subjected to stirring ranged from 90 to 100%, with a mean of 98.75%. When the effect of transport duration versus copepod survival was evaluated separately for each of the densities tested, significant correlations were obtained only for the highest densities: 80 and 120 ind.L. In both cases, a negative correlation was obtained, with a reduction in copepod survival as copepod density increased. A weak correlation was obtained for 80 ind.L ($R^2 = 0.23$) and a moderate for 120 ind.L ($R^2 = 0.57$) (Fig. 1).

When the effect of copepod density versus copepod survival was evaluated separately for each of the transport times tested, a significant correlation was obtained only for the longest time, 240 minutes. This was a negative and moderate correlation ($R^2 = 0.46$) with decreasing copepod survival rate as the transport time increased (Fig. 1).

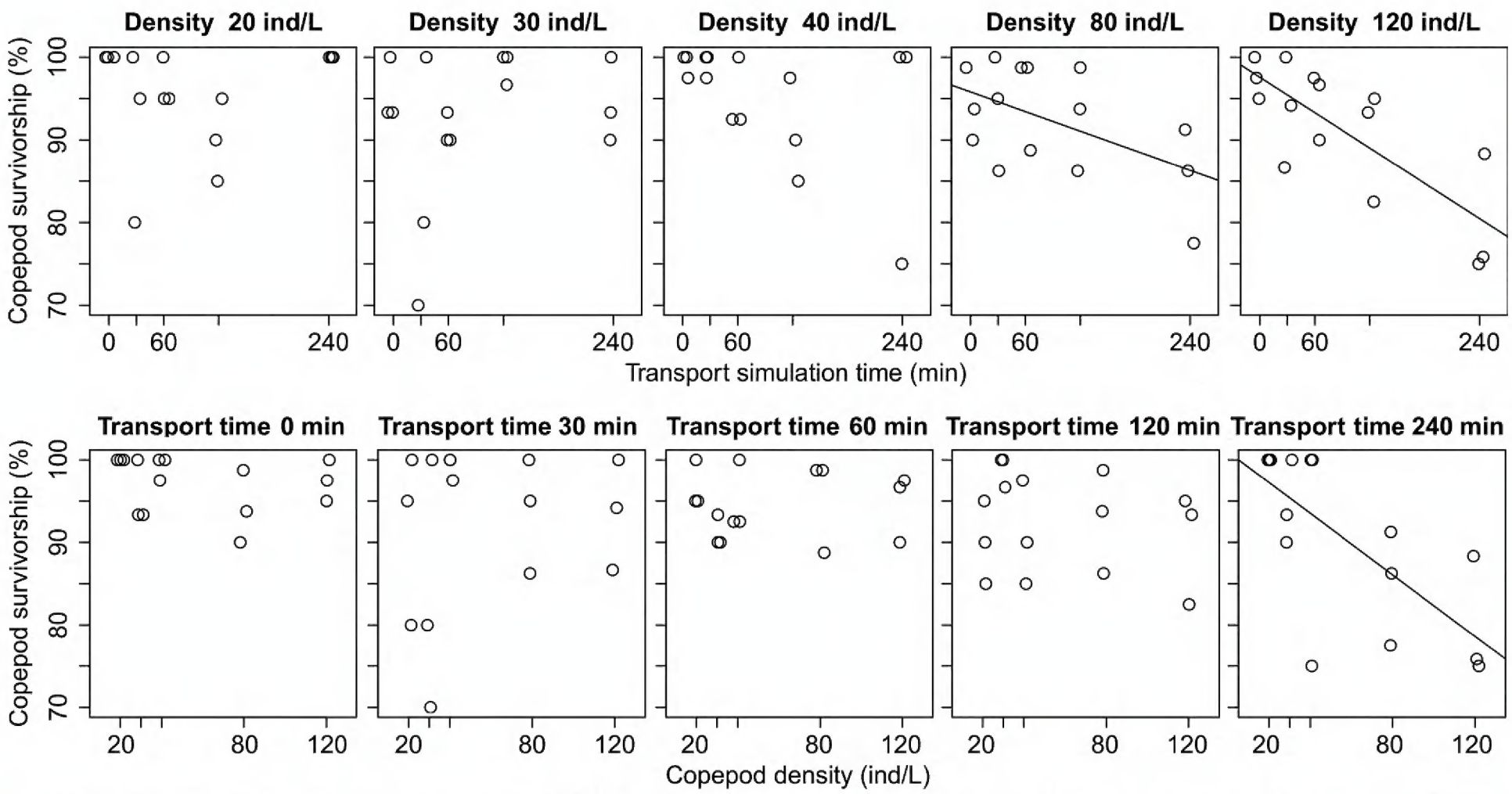
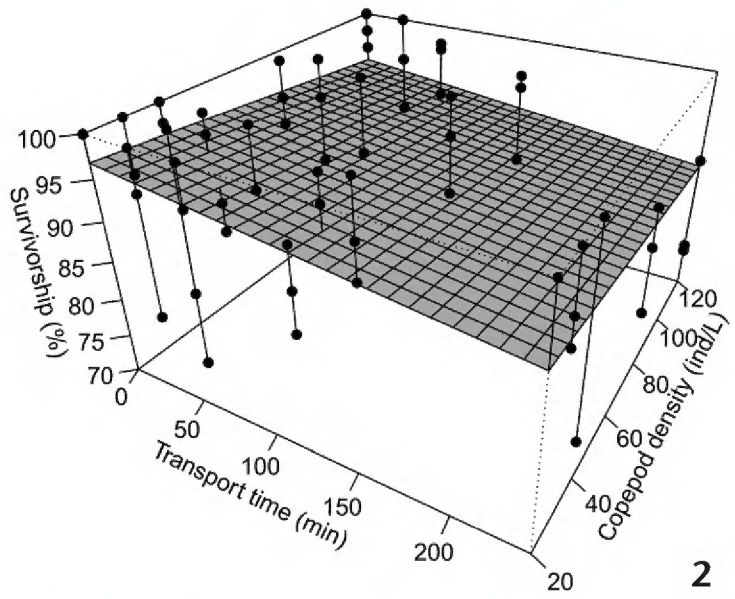


Figure 1. The dots represent the observations and the lines the linear regression models for significant correlations. The first row of graphs demonstrates the effect of transport time over copepod survival separately for different loading densities and the second demonstrates the effect of the loading density over the copepod survival, separately for different transport durations. Significant correlations: Density 80 ind.L^{-1} – Model: Copepod survival = 95.81 - 0.039*transport time (p-value = 0.038, Adjusted $R^2 = 0.23$); Density 120 ind.L^{-1} – Model: Copepod survival = 97.58 - 0.071*transport time (p-value = > 0.001, Adjusted $R^2 = 0.57$); Transport time 240 min – Model: Copepod survival = 100.97 - 0.186*copepod density (p-value = 0.0003, Adjusted $R^2 = 0.46$).



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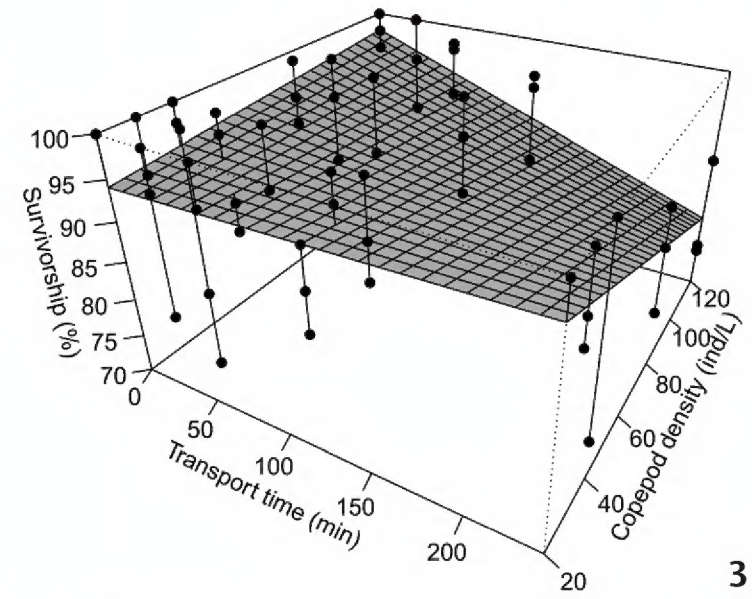

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Figures 2–3. The dots indicate the observations and the surface plot the multiple linear regression models not considering (2) and considering (3) the interaction between explanatory variables. Models: (2) Copepod survivorship = 97.65 - 0.023*transport time - 0.03*copepod density (p-value = 0.015, Adjusted R^2 = 0.084); (3) Copepod survivorship = 93.40 + 0.024*transport time + 0.035*copepod density - 0.0008*transport time*copepod density (p-value = > 0.001, Adjusted R^2 = 0.20).

When the combined effect of density and transport time were evaluated, the importance of the interaction between the two explanatory variables was evidenced. Significant correlations were obtained for both models considering and not considering this interaction (p-values of 0.015 and <0.001, respectively). The correlation was weak for both and the models explained 20% and 8.1%, respectively, of the variance in copepod survivorship. A higher R^2 (0.20) was obtained when the interaction was considered, compared to the model that disregarded the interaction between explanatory variables (R^2 = 0.08) (Figs 2, 3).

DISCUSSION

To our knowledge, this is the first study that investigated the survivorship of copepods under transport conditions. Similar studies on other crustacean species are available in the scientific literature, especially on the transport of younglings. Most of the studies indicated that crustaceans are very resistant to transport conditions.

In the case of the copepod *Penaeus monodon* (Fabricius, 1798), for example, the survival rate of post-larvae after eight hours of transport in a density of 600 ind.L was up to 95% (Hamid and Mardjono 1979). Smith and Ribelin (1984) concluded that post-larvae of the shrimp *Litopenaeus vannamei* (Boone, 1931) can be transported for up to 18 hours at a density of 190 ind.L with insignificant mortality. Additionally, Ventura et al. (2010) investigated the survival of *Ucides cordatus* Linnaeus, 1763 (Decapoda: Ocypodidae megalopae) and concluded that they can be transported at loading densities of 300 ind.L^{-1} during periods of six hours with minimal mortality. Only one of the reviewed studies reported lower survival rates. For the crab species *Scylla serrata* (Forskal, 1775), Quinitio and Parado-Estepa (2000) obtained 58% survivorship after six hours of transport simulation of megalopae in a density of 150 ind.L. The authors suggested the use of maximum densities of 50 ind.L for younglings of this species.

The present study tested loading densities of up to 120 ind.L under a maximum transport time of 240 minutes. The survivorship of *M. longisetus* was high, with 75% of the results equal or higher than 90% survival. The results also showed that the minimum mortality is expected for transport times up to 120 minutes and loading densities up to 40 ind.L. For higher densities or longer transport times, however, mortality seems to be significantly affected. Our results also indicated that the loading density and duration of the transport act synergistically in the reduction of survivorship of copepods. Future studies are important to test higher densities and longer transport times to confirm these tendencies.

Mortalities of at least 10%of the copepods were obtained for all the treatments, including the control groups. It is possible that the act of transferring copepods to the transport vials was at least in part responsible for this mortality. Future studies are needed to investigate ways to minimize the damage of the copepods during the transference to the transport vials.

Based on our results, it is possible to suggest that *M. longisetus* can be successfully transported from hatchery to target areas for the biological control of *A. aegypti* mosquitoes in densities up to 40 ind.L during 120 minutes with minimum mortality. This information can be useful to the establishment of transport strategies in programs for biological control of *A. aegypti*. More experiments are needed to find out how long these copepods can survive under transport conditions.

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